submission, Applicants have limited their claims to the subject matter for which indication of allowance has been provided as per the Interview Summary mailed February 7, 1996. Applicants anticipate that the application may now proceed to allowance, and would be pleased to confer with the Examiners concerning any remaining issues, such as further suggestions for claim language, as may be appropriate.

Please amend the above-referenced as follows.

In the Specification

On page 15, after line 26, please insert the following paragraph as the first paragraph of Example 5:

-- Initial attempts to assemble a full length CFTR coding sequence resulted in extensive rearrangements in the DNA clones obtained. This, together with the reported absence of full-length clones in the original isolates, made us consider whether transfected bacteria might be selecting against the full-length CFTR cDNA. Although the CFTR DNA sequence includes some repeated elements, they are not more frequent or extensive than is typical for other stable DNA sequences. This argued against instrinsic DNA instability as the cause of the problem but instead suggested that the presence of the full length cDNA was toxic to cells. Such toxicity generally results from the inappropriate expression

5'

of cDNA and could be due to the presence of cryptic promoters within the DNA sequence. --

On page 16, after line 10, please insert the following paragraphs (with an introductory subtitle) as the last section(s) of Example 5:

-- Identification of the Active Cryptic Promoter Site

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To search for any such transcription initiation sites, segments of CFTR cDNA were placed upstream from a promoterless gene encoding chloramphenicol acetyl transferase (CAT), transfected into *E. coli*, and the bacteria challenged with chloramphenicol. See Brosius, Gene, 27, 151-160 (1984). By this means an active promoter sequence was identified beginning at residue 908 in exon 6B (see Figure 14). This sequence shows good homology with the consensus E. coli promoter sequence, having 9 of 13 residues identical, including a highly conserved T residue at the 3' end of the -10 box (see Figure 14(b), and also Hawley et al., Nucleic Acids Research, 11, 2237-2255, 1983).

As shown in Figure 14, panels (a), (b), (c), a functional *E. coli* promoter was identified within the CFTR cDNA, and a complete CFTR open reading frame was assembled. Figure 14(a) shows the plasmid clones used to identify poromter sequences within the CFTR cDNA by activation of a promoterless CAT gene. *E. coli* cells containing the

constructs were challenged with increasing concentrations of chloramphenicol. Control plasmids contained either no insert (pKK232-8) or the beta galactosidase promoter (plac) from pBluescript SK$^-$ (Stratagene). Nucleotide coordinates for CFTR gene fragments are as given by Riordan et al., Science, 245, 1066-1073, (1989). Symbols in Figure 14(a) are: +, growth to confluence; +/-, slow growth; -, no growth.

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Figure 14(b) shows alignment of the promoter sequence within the CFTR cDNA with the consensus *E. coli* promoter sequence (see also Cohen et al., Proceedings of the National Academy of Sciences, USA, 70, 3240-3244, 1973.

Figure 14(c) shows a map of the low copy number plasmid pSC-CFTR-2, which contains the first 5.5 kilobases of the CFTR sequence of Riordan et al., Science, 245, 1066-1073, (1989), including the entire open reading frame. With respect to all of the above, see Gregory et al., Nature, 347, 382-386, (1990) for further details. --

At page ~~43,~~ after line 17, insert:

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-- Brosius, J., Gene, 27, 1984, 151-160.--

At page 44, after line 26, insert:

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-- Hawley, D. et al., Nucleic Acids Research, 11, 1983, 2237-2255 --